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RESEARCH ARTICLE (ORIGINAL)

Effects of chlorhexidine on the salivary microorganisms of patients in intensive care units

Efeito da clorexidina em microrganismos na saliva de doentes internados em unidade de terapia intensiva

Efecto de la clorhexidina en los microorganismos de la saliva de pacientes ingresados en una unidad de cuidados intensivos

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Abstract
Background: The control of oral biofilm with antiseptics should follow the protocols designed for patients in intensive care units (ICUs).
Objective: To assess the effects of using 0.12% chlorhexidine solution in reducing the number of microorganisms in the saliva of ICU patients.
Methodology: Cohort study with 45 volunteers divided into Medical Clinic (control group, oral self-care), non-intubated ICU, and intubated ICU. Oral hygiene in the ICU was performed with chlorhexidine. Microbiological analysis was performed through salivary bacterial count. Data were analyzed using ANOVA.
Results: The number of microorganisms decreased after oral hygiene. A significant difference was found between the control and the ICU intubated groups in the immediate period and after 30 minutes. The number of microorganisms in intubated patients decreased 12 hours after oral hygiene with chlorhexidine.
Conclusion: The number of oral microorganisms decreases in intubated patients 12 hours after oral hygiene with chlorhexidine. These results may contribute to elaborating oral hygiene protocols for ICUs and planning strategies, for example, to reduce the costs and the side effects associated with chlorhexidine use.

Keywords: oral hygiene; chlorhexidine; colony count, microbial; pneumonia, ventilator-associated; intensive care units

Resumo
Enquadramento: O controlo do biofilme oral com anti-sépticos deve ser adequado aos protocolos elaborados para os doentes na unidade de terapia intensiva (UTI).
Objetivo: Avaliar o efeito da solução de clorexidina 0,12% na redução de microrganismos na saliva de doentes de UTI.
Metodologia: Estudo coorte com 45 voluntários divididos em: Clínica Médica (controlo, auto higiene oral), UTI não-entubado e UTI entubado. A higiene oral na UTI foi realizada com clorexidina. A análise microbiológica foi realizada com a contagem de microrganismos na saliva. A análise dos dados foi feita com ANOVA.
Resultados: Houve uma redução dos microrganismos após higiene oral. O controlo apresentou diferença significativa com UTI entubado nos períodos imediato e após 30 min. Doentes entubados apresentaram redução dos microrganismos até 12 horas após higiene com clorexidina.
Conclusão: A higiene com clorexidina reduz em até 12 horas os microrganismos bucais de doentes entubados. Estes resultados podem contribuir para elaboração de protocolos de higiene oral em UTI, além de sustentar estratégias como redução nos custos e efeitos colaterais associados à clorexidina.

Palavras-chave: higiene oral; clorexidina; contagem de colônias microbiana; pneumonia associada à ventilação mecânica; unidades de terapia intensiva

Resumen
Marco contextual: El control de la biopelícula oral con antisépticos debe ser adecuado a los protocolos elaborados para los pacientes de una unidad de cuidados intensivos (UCI).
Objetivo: Evaluar el efecto de la solución de clorhexidina al 0,12% en la reducción de los microorganismos en la saliva de los pacientes en una UCI.
Metodología: Estudio de cohorte con 45 voluntarios divididos en Clínica Médica (control, autohigiene bucal), UCI no intubados y UCI intubados. La higiene bucal en la UCI se realizó con clorhexidina. El análisis microbiológico se realizó con el recuento de microorganismos en la saliva. El análisis de los datos se realizó con ANOVA.
Resultados: Hubo una reducción de los microorganismos después de la higiene bucal. El control mostró una diferencia significativa con los pacientes intubados en la UCI en el momento y después de 30 minutos. Los pacientes intubados mostraron una reducción de los microorganismos hasta 12 horas después de la higiene con clorhexidina.
Conclusión: La higiene con clorhexidina reduce en un máximo de 12 horas los microorganismos bucales en pacientes intubados. Estos resultados pueden contribuir a la elaboración de protocolos de higiene bucal en las UCI, además de apoyar estrategias como la reducción de costes y los efectos secundarios asociados a la clorhexidina.

Palabras clave: higiene bucal; clorhexidina; recuento de colonia microbiana; neumonía asociada al ventilador; unidades de cuidados intensivos

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Introduction

Hospital-acquired infections (HAIs) have been associated with the development of resistant microorganisms worldwide. Previous studies show that up to 50% of HAIs in intensive care units (ICUs) have resistant etiological agents (Despotovic et al., 2020). Patients admitted to ICUs are among those more likely to develop HAIs, representing a prevalence rate up to 10 times higher when compared to the prevalence rates of other patients admitted to other hospital units. HAIs increase morbidity and mortality rates, length of hospital stay, and costs related to healthcare services (Sabino et al., 2020).

The ICU mortality rate due to HAIs is 70%, which is a much higher percentage than in other causes of death related to critically ill patients in these units (Despotovic et al., 2020). The strategies for preventing HAIs are strongly supported by scientific evidence. The use of prevention measures and the high prevalence rates are a major challenge for professionals (Junior et al. 2020). One of these measures is dental practices, and one of their main focuses is the prevention of ventilator-associated pneumonia (VAP), which is one of the most common infections affecting critically ill patients (Klompas, 2019). The prevalence of VAP in intubated patients is 9%-27%, and the mortality rate can exceed 50% in mechanically ventilated patients, with a progressive increase as intubation time increases (Galhardo et al., 2020; McCue & Palmer, 2019).

Thus, oral biofilm control through oral hygiene with antiseptics is one of the main mechanisms for preventing and reducing VAP, and chlorhexidine is the most suitable agent (Galhardo et al., 2020) because it is a first-choice oral antiseptic in dental biofilm control. Oral hygiene with chlorhexidine is recommended for mechanically ventilated patients because of its demonstrated benefits and association with VAP reduction (Guerra et al., 2019). However, the time of action of this drug may change in patients admitted to the ICU.

Considering the lack of a protocol about oral hygiene in ICUs, this study aims to assess the effects of using 0.12% chlorhexidine solution in reducing microorganisms in the saliva of ICU patients.

Background

The interventions of multidisciplinary teams in the assessment, oral hygiene, and specific preventive procedures using antiseptics are important for the patient's overall health (Junior et al., 2020). Oral hygiene with antiseptic solutions is a dental measure to prevent HAIs. Chlorhexidine is the most effective substance in controlling biofilm due to its broad antimicrobial spectrum and substantivity, remaining for up to 12 hours in the oral cavity because of its gradual release (Galhardo et al., 2020).

For this reason, mechanically-ventilated patients in ICUs have significantly low mortality rates when oral hygiene is performed with a chlorhexidine solution (Klompas, 2019). However, it is suggested that the retention time of this substance in the oral cavity of mechanically-ventilated in patients may differ due to several factors inherent to these patients' critical condition (Rabello et al., 2018).

Given the relevance of this topic, the critical condition of ICU patients is a challenge for health professions, for which reason it is important to use evidence-based protocols focused on preventive strategies.

Research question

What are the effects of a 0.12% chlorhexidine solution in reducing microorganisms in the saliva of ICU patients?

Methodology

This cohort study was carried out in a university hospital in the state of Paraná (southern region of Brazil) with volunteers admitted to the ICU and medical clinic. These patients were divided into three groups (n = 15/group): control group (Medical Clinic), Non-intubated ICU, and Intubated ICU (Figure 1).

Patients admitted to the medical clinic (control group) were able to perform their oral hygiene (brushing with toothpaste and rinsing with antiseptic solution). This group was defined as the control group because its participants had the motor ability to perform their daily activities independently. ICU groups consisted of intubated patients on mechanical ventilation (orotracheal intubation) or non-intubated patients (spontaneous ventilation).

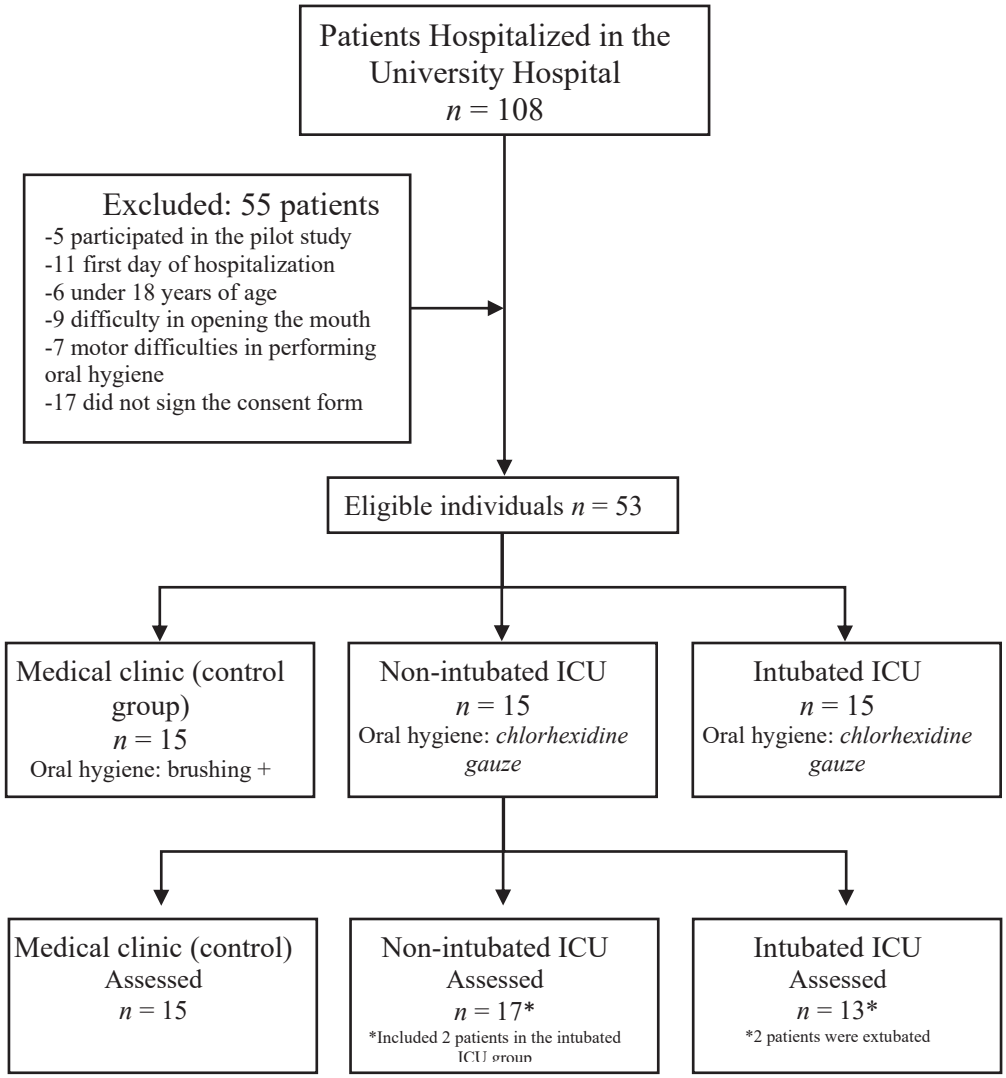
The Institutional Ethics Committee approved the research study (CAAE: 44726615.9.0000.0105). All participants in the medical clinic group and the persons responsible for ICU patients (several levels of consciousness) signed the informed consent form. Data were collected between September and December 2015.

Data from a pilot study (unpublished) with five patients admitted to the ICU were used for sample calculation, considering microbial count in different periods. An effect size of 0.88 was obtained with the mean and the standard deviation. Thus, a sample of 37 volunteers was estimated for a power of 87%. Sample size was increased by 20% to compensate for possible losses in the volunteers' follow-up, with a final sample of 45 individuals (G*Power, version 3.1.9.2).

The study included individuals over 18 years of age who had been hospitalized for more than 2 days. The exclusion criteria were as follows: patients unable to open the mouth, patients in the medical clinic with poor self-care skills (diagnosis obtained by the nursing team), individuals with a history of adverse reaction to chlorhexidine or other formula components, and patients/guardians who did not sign the informed consent form.

Figure 1

Study flowchart



Note. *patients extubated two hours after the beginning of the study due to clinical improvement, adequate oxygenation, and hemodynamic stability. They were allocated to the non-intubated ICU group.

The following parameters were used to characterize aspects related to the oral cavity: mucosal condition (analyzed through a systematic sequence for the detection of color, texture, and surface changes), contour and size (buccal mucosa, labial mucosa, hard and soft palate, gingiva, tonsillar pillars, tongue, floor of mouth, and visible oropharynx); number of teeth; and oral hygiene. Hyposalivation was assessed through the dryness of the mucous membranes using a wooden spatula, checking for adherence upon removal (buccal mucosa and dorsum of tongue) and moistening of the spatula after removal from the oral cavity (Das & Challacombe, 2016). All clinical assessments were carried out using a flashlight (white LED light), gauze, and a wooden spatula. A dentist-surgeon performed the oral hygiene protocol using 5 ml of 0.12% chlorhexidine solution. Saliva samples were collected before, immediately after, 30 min, 3 hr, 12 hr, 24 hr, and 36 h after oral hygiene. Oral evaluation parameters were reevaluated at each time segment, along with the saliva collection. Between the experimental times, oral hygiene was performed with a gauze pad moistened with sterile 0.9% sodium chloride solution, at the times established in the hospital protocol (every 12 hours) to avoid harming the patient's general health during hospitalization. The control group performed a mouth rinse with 5 ml of 0.12% chlorhexidine for 1 minute and continued with the usual brushing during hospitalization. Saliva was collected from the lingual region (dorsum of the tongue) and the buccal mucosa using a sterile swab. The swab with the sample was submerged in 3 ml of sterile phosphate-buffered saline (PBS) and stored for microbiological laboratory analysis. The microbiological evaluation was performed through the total bacteria count. To this end, the 3 ml PBS solution, containing the swab used for collection, was homogenized in a shaker for 60 seconds, diluted in 10^{-1}, 10^{-2}, and 10^{-3}, and 100 µl of each solution,

and then cultivated on a Mueller-Hinton Agar Petri dish (20x100 mm). Subsequently, the dishes were stored in an oven at constant temperature (36.5 ºC) for 24 hours to count the number of colony-forming units per milliliter (CFU/ml).

ICU patients (non-intubated and intubated) were grouped for the analysis of qualitative variables: gender, mucosal condition (buccal mucosa, labial mucosa, hard and soft palate, gingiva, tonsillar pillars, tongue, floor of mouth, and oropharynx), hyposalivation (dryness of the mucosa), and oral hygiene. The Chi-square test was applied. For analysis of quantitative variables, data normality was tested with the Kolmogorov-Smirnov test. The counting of the number of CFU/ml in saliva was not normally distributed ($p < 0.05$), so the data were logarithmically transformed. Thus, the quantitative variables (age, number of teeth, and CFU count) were tested with ANOVA and post-hoc Tukey's test. The different analysis periods within the same group were compared using ANOVA for repeated measures and the post-hoc Tukey's test. The significance level was 5% (IBM SPSS Statistics, version 21.0).

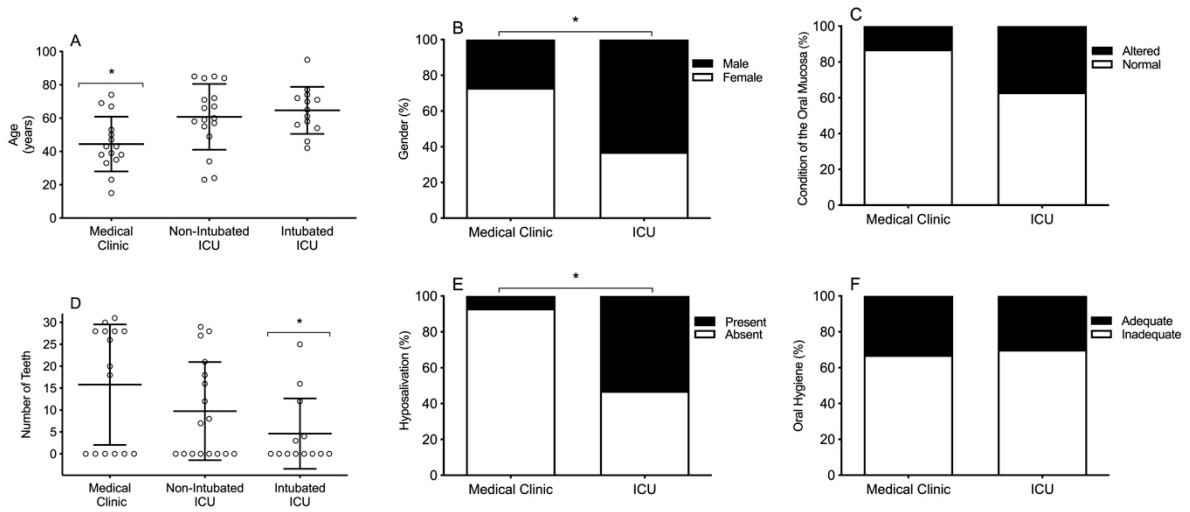
Results

All patients were followed up during the study. Only two from the intubated ICU group were reallocated to the non-intubated ICU group because they were extubated 2 hours after the beginning of the study.

Figure 2 shows the characteristics of the groups. A significant difference ($p < 0.05$) was found between the medical clinic volunteers (control group) and ICU patients regarding age, with the ICU patients showing the highest mean ages. The intubated ICU group had fewer teeth than the control group ($p = 0.035$). Hyposalivation was observed more frequently in ICU patients ($p = 0.002$).

Figure 2

Characteristics of patients hospitalized in the medical clinic (n = 15), non-intubated ICU (n = 17), and intubated ICU (n = 13)

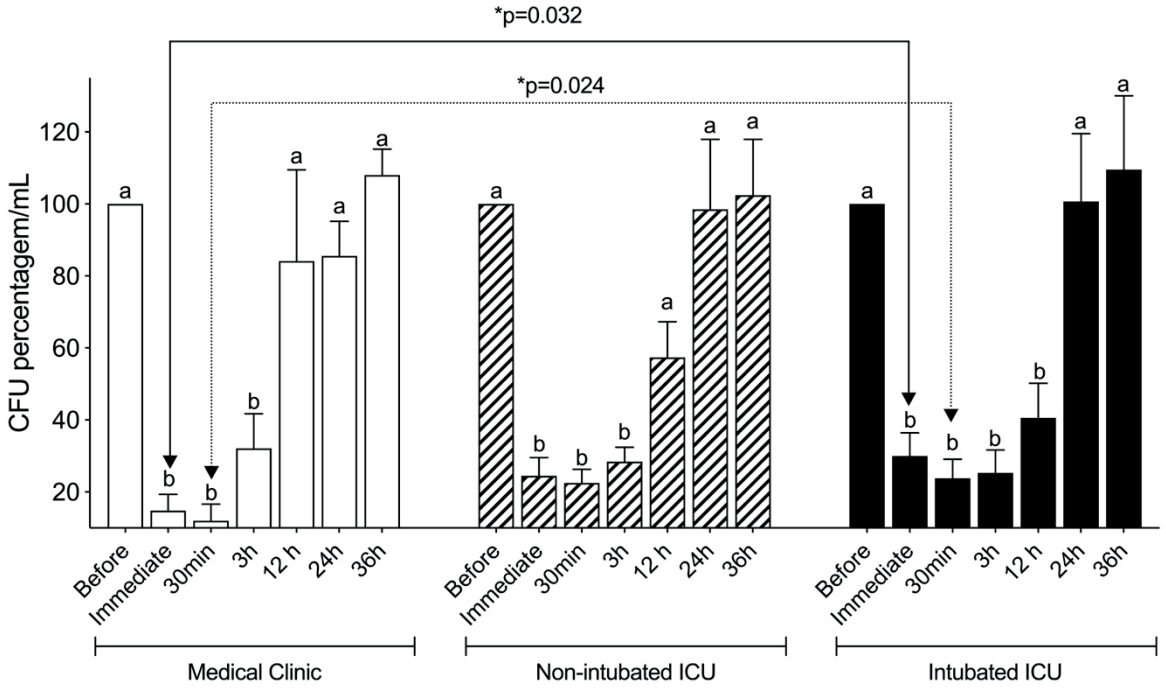


Note. (A) Patients' age, (*) significant difference with non-intubated ICU ($p = 0.028$) and intubated ICU ($p=0.009$). (B) Percentage distribution according to gender, (*) significant difference ($p = 0.020$). (C) Condition of the oral mucosa, non-significant difference ($p = 0.104$). (D) Number of patients' teeth according to the groups, (*) significant difference with intubated ICU ($p = 0.034$). (E) Hyposalivation, (*) significant difference ($p = 0.002$). Condition of oral hygiene, non-significant difference ($p = 0.820$). (A) and (D), points represent each patient, lines represent the mean and standard deviation, ANOVA with post-hoc Tukey test. (B), (C), (E) and (F), Chi-square test.

Figure 3 shows the salivary bacteria count (percentage of CFUs) at different times. The number of microorganisms decreased in all groups after oral hygiene with a chlorhexidine solution. A significant difference was found between the control and intubated ICU groups in the immediate period (p = 0.042) and after 30 minutes (p = 0.030). Microbial reduction was higher in the control group than in the ICU patients. Comparison at different times within the same group showed a significant (p < 0.05) microbial reduction 3 hours after chlorhexidine use in the control and non-intubated ICU groups. In the intubated ICU group, microbial reduction was significant (p < 0.05) up to 12 hours after oral hygiene.

Figure 3

Mean and standard error of the percentage of colony-forming units (CFUs)/ml of saliva in the patients hospitalized in the Medical Clinic (n = 15), non-intubated ICU (n = 17), and intubated ICU (n=13) before and after the oral hygiene with a 0.12% chlorhexidine solution



Note. Group comparison in each period, (*) significant difference (p < 0.05), ANOVA with post-hoc Tukey test. Comparison of the different moments within the same groups. Different letters indicate a significant difference (p < 0.05) between the several moments (ANOVA for repeated measures with post-hoc Tukey test).

Discussion

The results revealed a decrease in bacterial counts up to 12 hours after oral hygiene with a 0.12% chlorhexidine solution in intubated ICU patients. This result shows that 62% of the microorganisms were eliminated in the first 12 hours after oral hygiene in intubated patients. All groups showed a decrease immediately after oral hygiene, demonstrating the antimicrobial effect of the 0.12% chlorhexidine solution associated with the mechanical action. Volunteers admitted to the medical clinic, who had the motor ability to perform their oral hygiene, and non-intubated ICU patients showed a decrease in the number of bacteria 3 hours after oral hygiene, representing a 70% reduction in oral bacteria. After 12 hours, the number of microorganisms decreased by 12% and 58% in both groups, respectively.

Chlorhexidine is widely used for reducing the number of microorganisms in the oral cavity, and several studies have reported its influence on the prevention of VAP in hospitalized patients (Camargo et al., 2019; Deschepper et al., 2018; Meidani et al., 2018; Rabello et al., 2018; Vidal et al., 2017; Zand et al., 2017). In this context, the time necessary for microbial reduction after oral hygiene with a 0.12% chlorhexidine solution is still unclear, especially in patients with an orotracheal tube. Variations in studies with different populations, concentrations, chlorhexidine preparations, and hygiene frequency make it difficult to compare different findings (Galhardo et al., 2020; McCue & Palmer, 2019; Rabello et al., 2018; Zand et al., 2017). Studies show that chlorhexidine did not reduce the total number of bacteria in the oral cavity after oral hygiene in mechanically-ventilated patients (Vidal et al., 2017; Scannapieco et al., 2009). The divergences with the results of this study are related to differences in the methods for

collecting materials (Scannapieco et al., 2009).
Previous studies support the suggested mechanism for microbial reduction in shorter time in Medical Clinic patients and non-intubated ICU patients than in intubated ICU patients. First, the literature reports that teeth are an important reservoir for microorganisms (Camargo et al., 2019; Vidal et al., 2017). In this study, the majority of intubated ICU patients had no teeth. Consequently, the increased number of bacteria in the teeth of the Medical Clinic and the non-intubated ICU volunteers may have influenced CFU count. In the Medical Clinic patients who were able to brush their teeth, the use of other substances, such as food and toothpaste, may have interacted with chlorhexidine, reducing the action time of the antimicrobial solution used (Kolahi & Soolari, 2006). Another possible reason for the reduced time in non-intubated ICU and Medical Clinic patients is that they could talk and chew, and these extrinsic factors reduce the substantivity of chlorhexidine, which is eliminated faster (Tomás et al., 2010).
Age and gender characteristics show that the highest mean ages were found in ICU patients, together with a prevalence of the male gender, which is in line with the literature (Deschepper et al., 2018; Meidani et al., 2018). The Medical Clinic volunteers had normal salivary flow, which also influences the elimination of chlorhexidine in the oral cavity. In contrast, in intubated ICU patients, chlorhexidine substantivity lasted 12 hours, explained by the high prevalence of hyposalivation, fewer teeth, and reduced motor skills.
Based on the results concerning the time for microbial reduction after oral hygiene with chlorhexidine, the aspects associated with the costs and the impact of the strategies should be considered to improve the effectiveness of hygiene protocols. Oral hygiene with chlorhexidine reduces VAP, reducing material, staff, medication, and structural costs. Thus, it is important to include this practice in hospital protocols. The ICU is the health area that receives and demands more financial resources, with an estimated 20% of the hospital's total expenses (Kyeremanteng et al., 2018). European studies show that the cost in the ICU per day is €1,168 to €2,025. In 2016, the reported cost in the UK was £1,738. In 2015, the cost of mechanical ventilation per day was, on average, £1,863. More specifically, the cost of VAP was £61 per patient/day (Saunders & Geogopoulos 2018). This study took place at a hospital where the recommended protocol is oral hygiene with 0.12% chlorhexidine solution four times/day. Based on data from intubated ICU patients, chlorhexidine exhibited a 12-hour substantivity, suggesting that oral hygiene with chlorhexidine twice a day is efficient and reduces product costs by 50% per day, VAP, and, consequently, all costs of intubated patients.
The authors of this study followed the hospital's standard protocol for oral hygiene. During the experimental period, chlorhexidine was used only in the first oral hygiene moment, and a sterile saline-soaked gauze was used in the other moments to avoid worsening the patients' overall health status. This oral hygiene method applied only once was used in a previous study to investigate the number of bacteria after oral hygiene at several time intervals (Scannapieco et al., 2009). The total reduction of microorganisms was checked using the CFU/ml analysis, a well-established method to investigate the number of bacteria (Zand et al., 2017). Thus, reducing the time interval between the moments of oral hygiene with chlorhexidine is a recommended strategy, as long as the procedure is performed correctly.
The main limitations of this study are its single-center cohort design, which may limit generalization, and the difficulty in creating a control group for comparison between patients. The Medical Clinic volunteers, who could perform their own hygiene, are related to variables such as using different brushing techniques and product brands, such as toothpaste, which may have influenced or interacted with chlorhexidine and, consequently, interfered in the results.
One of the most relevant aspects of this topic is the reduction in mortality rates and adverse effects. Chlorhexidine can cause some adverse effects such as mucosal ulceration, staining of the teeth, tongue, and restorations, and altered taste sensations (Guerra et al., 2019; Zand et al., 2017). Halving the number of times oral hygiene is performed with chlorhexidine in intubated patients may also reduce the number of adverse effects. Therefore, the results of this study allow for the development of future studies and increase the possibility of optimizing strategies to reduce and prevent HAIs, especially VAP, and, consequently, improve the quality of hospital care.

Conclusion

The results showed that oral hygiene with chlorhexidine reduces the number of microorganisms in the oral cavity of intubated ICU patients up to 12 hours. A microbial reduction was also observed in non-intubated ICU patients and volunteers from the medical clinic, which is maintained for up to 3 hours after using the chlorhexidine solution. Thus, these results can contribute to the development of protocols for using 0.12% chlorhexidine solution in ICU patients, which can become a strategy for reducing hospital costs and side effects associated with chlorhexidine use.

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